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Specification, as originally filed, with Application for Patent Serial No: **2,283,975**, on September 28, 1999, by **VASOGEN INC.**, for "Combined Therapies for Atherosclerosis Treatment".

PRIORITY DOCUMENT

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ABSTRACT OF THE DISCLOSURE

There is provided a combination treatment for slowing or arresting the progression and/or effecting the regression of atherosclerotic plaque deposits in a mammalian patient, said combination treatment including the administration to the patient of a cholesterol lowering drug such as a statin, and the administration to the patient of an aliquot of a patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light.

COMBINATION THERAPIES FOR TREATING ATHEROSCLEROSIS IN MAMMALS

FIELD OF THE INVENTION

This invention relates to compositions and procedures for treatment of elevated lipid levels in the serum of mammalian patients, and for treatment of atherosclerosis and cardiovascular disorders associated therewith or resulting therefrom.

BACKGROUND OF THE INVENTION

Hyperlipidemias such as hypercholesterolemia and elevated serum triglyceride levels are among the most potent risk factors in the causation of atherosclerosis, which is the build-up of fatty plaque deposits within the walls of blood vessels. For example, high levels of serum cholesterol bound to low density lipoprotein (LDL), intermediate density lipoprotein (IDL) or very low density lipoprotein (VLDL) are known to correlate strongly with the occurrence of atherosclerosis in humans. In particular, it is known that the higher the circulating levels of cholesterol in the form of LDL, IDL and VLDL cholesterol, and the higher the circulating levels of other lipids such as triglycerides, the more likely it is that cholesterol and lipids will be deposited within the blood vessel wall and cause or contribute to atherosclerosis.

In hypercholesterolemia, for example, the increase in the blood cholesterol level is associated mainly with a rise in the concentration of LDL, IDL and VLDL cholesterol. However, the specific causes of hypercholesterolemia are complicated and varied. At least one kind of hypercholesterolemia, known as familial hypercholesterolemia, is caused by a mutation in the gene for the LDL receptor that moves cholesterol out of the

blood, primarily in the liver. Much more commonly, hypercholesterolemia has been associated with genetic factors and high dietary intake of saturated fatty acids and cholesterol, resulting in elevated blood cholesterol levels. High serum triglyceride levels have also been associated with high dietary intake of fatty acids.

Reduction of hyperlipidemia, including hypercholesterolemia, results in a delayed onset of atherosclerosis and a decrease in the progression of atherosclerosis, thus reducing the risk of coronary heart disease. Some forms of hyperlipidemia, including hypercholesterolemia, are potentially partially reversible with current techniques of preventive management. Taking cholesterol-lowering drugs can result in a reduction in serum cholesterol, and other drugs may lower serum triglyceride levels. Dietary therapy is usually recommended for all patients with hyperlipidemia but the effect is often not sufficient to reduce risk optimally.

A wide variety of cholesterol-lowering drugs are available on the prescription drug market, and are widely prescribed. These include the so-called "statin" drugs (lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin and cerivastatin), which are generally known as HMG-CoA reductase inhibitors, since their mechanism of action is understood to be through the selective , competitive inhibition of the hepatic enzyme 3-hydroxy-3-methylglutaryl-co-enzyme A reductase. In some cases, e.g. simvastatin, the active molecule is a metabolite formed in the body after ingestion of the drug by the patient. In other cases, the administered drug (commonly a pharmaceutically acceptable salt) is itself the active molecule. Other classes of cholesterol-lowering drugs include the bile acid sequestrants (cholestyramine resin, colestipol hydrochloride); fibrates (bezafibrate, clofibrate, fenofibrate and gemfibrozil: niacin derivatives (niacin and xanthinol niacinate); and other miscellaneous compounds such as dextrothyroxine. All of these, but especially the statin drugs, have proved successful in reducing serum cholesterol levels in

mammalian patients, so as to attack one of the underlying causes of the development of atherosclerosis.

Atherosclerosis, the build-up of fatty plaque deposits within the walls of blood vessels, commonly develops over a relatively lengthy period of time in patients. To-date, treatments such as diet adjustment and administration of cholesterol lowering drugs have slowed or even halted the development of atherosclerosis, but only limited success has been reported for causing regression of atherosclerosis, i.e. diminution of the atherosclerotic fatty plaque deposits. The need for procedures and compositions which will not only reduce serum lipid levels, especially cholesterol levels, in mammalian patients, but will also inhibit development and effect regression of atherosclerotic plaque deposits, is clearly apparent.

SUMMARY OF THE INVENTION

An aspect of the invention is the provision of a combination treatment for slowing or arresting the progression and/or effecting the regression of atherosclerotic plaque deposits in a mammalian patient, said combination treatment including the administration to the patient of a cholesterol lowering drug and the administration to the patient of an aliquot of a patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light.

Another aspect of the invention comprises the use of an aliquot of a patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light; and a cholesterol-lowering drug, for reducing serum lipid levels and/or combating development of atherosclerosis in a mammalian patient.

A further aspect is a process for enhancing the reduction of

serum lipid levels in a mammalian patient caused by administration of a cholesterol-lowering drug, which comprises administering to the patient an aliquot of the patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light and administering to the patient a cholesterol-lowering drug.

DESCRIPTION OF THE PREFERRED EMBODIMENTS.

The preferred cholesterol-lowering drugs for use in the present invention are the statin drugs referred to above, namely lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin and cerivastatin. However, other cholesterol-lowering drugs as mentioned above may also be used. To be effective in the present invention, they can be administered in dosages previously recommended and used when the drugs are administered alone, and by the same routes of administration. They can be administered over the same time periods over which the patient is undergoing administration of the modified blood aliquots, as described below. The administration of the cholesterol-lowering drug can precede the commencement of the patient's treatments with modified blood aliquots and can continue thereafter, if required. The modified blood treatments may precede the commencement of the administration of the statin drug, and conclude before its commencement. Modified blood treatment may alternate with cholesterol drug administration periods, overlap therewith or totally coincide therewith.

The aliquot of blood used as a part of the combination therapy in the present invention is an aliquot of the patient's own blood which has been extracorporeally treated by being subjected to one or more stressors which have been found to modify the blood. The blood aliquot can be modified by subjecting the blood, or separated cellular or non-cellular fractions of the blood, or mixtures of the separated cells and/or non-cellular fractions of the blood, to stressors selected from thermal stress, ultraviolet light and oxidative

environments such as treatment with ozone/oxygen mixtures, or any combination of such stressors, simultaneously or sequentially.

Effects of the treatment according to the present invention is a substantial reduction of lipid levels in serum and deposition of lipids within blood vessel walls, a retardation of the progression of plaque deposition, and in some cases to cause existing plaques to regress. It is believed that this observed vessel protection is due at least in part to the reduced serum lipid levels in subjects treated by the method of the present invention. However, the reduced deposition of lipids within blood vessel walls may also occur in the absence of a reduction in serum lipids. Synergistic interaction between the components of the combination therapy of the present invention may be observed in the rate or degree of serum lipid lowering, and/or in the rate or degree of atherosclerotic plaque deposition, and/or in the rate or degree of atherosclerotic plaque regression, and/or in the rate or degree of improvement in plaque stabilization. Anti-inflammatory action of the combined therapy, especially as derived from the treated blood ingredient of the combination, which is believed to have the effect of down-regulating the activity of the Th-1 component of the T-cells present in the blood, responsible for secretion of inflammatory cytokines such as IL-2, IFN-γ and TNF-α, and/or up-regulation of the Th-2 component of T-cells responsible for secretion of anti-inflammatory cytokines such as IL-10 or IL-4, is postulated to be at least part of an underlying mechanism of the success of the combination therapy in reducing atherosclerotic plaque.

The combination therapy of the invention can be administered not only to treat symptoms of atherosclerosis, but also as a preventative for patients at risk of development of atherosclerosis, and as a preventative in combating of cardiovascular disease.

The effect of the stressors is to modify the blood, and/or the

cellular or non-cellular fractions thereof, contained in the aliquot. The modified aliquot is then re-introduced into the subject's body by any suitable method, preferably selected from intra-arterial injection, intramuscular injection, intravenous injection, subcutaneous injection, intraperitoneal injection.

The preparation and use of such modified autologous blood aliquots in treatment of, inter alia, peripheral vascular disease has been previously disclosed in U.S. patent 5,591,457 Bolton. The disclosure of that patent is incorporated herein by reference, in its entirety.

The stressors to which the aliquot of blood is subjected <u>ex vivo</u> according to the method of the present invention are selected from temperature stress (blood temperature above or below body temperature), an oxidative environment and ultraviolet light, individually or in any combination, simultaneously or sequentially. Suitably, in human subjects, the aliquot has a volume sufficient that, when re-introduced into the subject's body and in the presence of the cholesterol-lowering drug in the body, a reduction in a serum lipid level and/or a retardation in progression or a regression of atherosclerotic plaque formation is achieved in the subject. Preferably, the volume of the aliquot is up to about 400 ml, preferably from about 0.1 to about 100 ml, more preferably from about 5 to about 15 ml, even more preferably from about 8 to about 12 ml, and most preferably about 10 ml.

It is preferred, according to the invention, to apply all three of the aforementioned stressors simultaneously to the aliquot under treatment, in order to ensure the appropriate modification to the blood. It may also be preferred in some embodiments of the invention to apply any two of the above stressors, for example to apply temperature stress and oxidative stress, temperature stress and ultraviolet light, or ultraviolet light and oxidative stress. Care must be taken to utilize an appropriate level of the stressors to thereby effectively modify the blood to achieve an effect on the atherosclerotic

condition.

The temperature stressor warms the aliquot being treated to a temperature above normal body temperature or cools the aliquot below normal body temperature. The temperature is selected so that the temperature stressor does not cause excessive hemolysis in the blood contained in the aliquot and so that, when the treated aliquot is injected into a subject, a lipid reduction and/or a retardation in progression or regression in the formation of atherosclerotic plaque will be achieved. Preferably, the temperature stressor is applied so that the temperature of all or a part of the aliquot is up to about 55°C, and more preferably in the range of from about -5°C to about 55°C.

In some preferred embodiments of the invention, the temperature of the aliquot is raised above normal body temperature, such that the mean temperature of the aliquot does not exceed a temperature of about 55°C, more preferably from about 40°C to about 50°C, even more preferably from about 40°C to about 44°C, and most preferably about 42.5 ± 1°C.

In other preferred embodiments, the aliquot is cooled below normal body temperature such that the mean temperature of the aliquot is within the range of from about -5°C to about 36.5°C, even more preferably from about 10°C to about 30°C, and even more preferably from about 15°C to about 25°C.

The oxidative environment stressor can be the application to the aliquot of solid, liquid or gaseous oxidizing agents. Preferably, it involves exposing the aliquot to a mixture of medical grade oxygen and ozone gas, most preferably by bubbling through the aliquot, at the aforementioned temperature range, a stream of medical grade oxygen gas having ozone as a minor component therein. The ozone content of the gas stream and the flow rate of the gas stream are preferably selected such that the amount of ozone

introduced to the blood aliquot, either on its own or in combination with other stressors, does not give rise to excessive levels of cell damage such that the therapy is rendered ineffective. Suitably, the gas stream has an ozone content of up to about 300 μg/ml, preferably up to about 100 μg/ml, more preferably about 30 μg/ml, even more preferably up to about 20 μg/ml, particularly preferably from about 5 μg/ml to about 20 μg/ml, and most preferably about 14.5 ± 1.0 μg/ml. The gas stream is suitably supplied to the aliquot at a rate of up to about 2.0 litres/min, preferably up to about 0.5 litres/min, more preferably up to about 0.4 litres/min, even more preferably up to about 0.33 litres/min, and most preferably about 0.24 ± 0.024 litres/min. The lower limit of the flow rate of the gas stream is preferably not lower than 0.01 litres/min, more preferably not lower than 0.1 litres/min, and even more preferably not lower than 0.2 litres/min.

The ultraviolet light stressor is suitably applied by irradiating the aliquot under treatment from a source of UV light while the aliquot is maintained at the aforementioned temperature and while the oxygen/ozone gaseous mixture is being bubbled through the aliquot. Preferred UV sources are UV lamps emitting UV-C band wavelengths, i.e. at wavelengths shorter than about 280 nm. Ultraviolet light corresponding to standard UV-A (wavelengths from about 315 to about 400 nm) and UV-B (wavelengths from about 280 to about 315) sources can also be used, either alone or in combination with each other and UV-C sources. For example, an appropriate dosage of such UV light, applied simultaneously with the aforementioned temperature and oxidative environment stressors, can be obtained from lamps arranged to surround the sample container holding the aliquot, operated at an intensity to deliver a total UV light energy at the surface of the blood of from about 0.025 to about 10 joules/cm^2, preferably from about 0.1 to about 3.0 joules/cm^2, may advantageously be used. Preferably, four such lamps are used.

The time for which the aliquot is subjected to the stressors is

normally within the time range of up to about 60 minutes. The time depends to some extent upon the chosen intensity of the UV light, the temperature, the concentration of the oxidizing agent and the rate at which it is supplied to the aliquot. Some experimentation to establish optimum times may be necessary on the part of the operator, once the other stressor levels have been set. Under most stressor conditions, preferred times will be in the approximate range of from about 2 to about 5 minutes, more preferably about 3 minutes. The starting blood temperature, and the rate at which it can be warmed or cooled to a predetermined temperature, tends to vary from subject to subject. Such a treatment provides a modified blood aliquot which is ready for injection into the subject.

For use in the process of the present invention, the blood aliquot may be treated with the stressors using an apparatus of the type described in U.S. Patent No. 4,968,483 to Mueller. The aliquot is placed in a suitable, sterile, UV light-transmissive container, which is fitted into the machine. The UV lamps are switched on for a fixed period before the gas flow is applied to the aliquot providing the oxidative stress, to allow the output of the UV lamps to stabilize. The UV lamps are typically on while the temperature of the aliquot is adjusted to the predetermined value, e.g. 42.5 ± 1 °C. Then the oxygen/ozone gas mixture, of known composition and controlled flow rate, is applied to the aliquot, for the predetermined duration of up to about 60 minutes, preferably 2 to 5 minutes and most preferably about 3 minutes as discussed above, so that the aliquot experiences all three stressors simultaneously. In this way, blood is appropriately modified according to the present invention to achieve the desired effects.

A patient preferably undergoes a course of treatments of removal of a blood aliquot, treatment thereof as described above and re-administration of the treated blood to the patient, with the cholesterol-lowering drug being administered as separate doses during this course of treatments. Such a

course may be a daily treatment for 4-6 days, followed by an interval and then a second course of daily treatments for 4-6 days. A preferred dosage regimen for the treated blood portion of the combination therapy is the administration of from 2-4 aliquots of autologous blood treated with stressors extracorporeally as described above, with the administration of any pair of consecutive aliquots being either on consecutive days , or being separated by a rest period of from 1-21 days on which no aliquots are administered to the patient, the rest period separating one selected pair of consecutive aliquots being from about 3 - 15 days, A more specific, preferred dosage regimen would be a total of three treatments and aliquots, with the first and second aliquots being administered on consecutive days and a rest period of 11 days being provided, between the administration of the second and third aliquots. The combination therapy of the invention may be useful in treatment of hypercholestolemia resulting from all the various aforementioned causes.

Statin drug administration may be at a lower than normal dosage during the treated blood administration course, or at the same level of dosage to obtain a faster plaque regression rate. The patient may have been taking the statin drug for a period of time before adopting the combination therapy of the invention, and may continue on the statin drug, at the same or at a reduced dosage level, following the termination of the combination therapy. The combination therapy, with further courses of blood aliquot removal, treatment and re-administration, may be repeated at intervals, of, say, 3 -12 months, in the event that the effectiveness of the previous combination therapy wears off or that the continued administration of statin drug alone is insufficiently effective.

The combination therapy of the invention is believed to affect the endothelium in such a way that it is rendered more resistant to the passage and deposition of the aforementioned lipid fractions.

Suitable daily dosages of statin drugs for human patients in the combination therapy of the present invention are, as noted, generally in accordance with those previously used when statin drugs are administered alone. These are, in respect of atorvastatin, simvastatin, lovastatin, fluvastatin and pravastatin, from about 5 mg to about 200mg daily, for an adult of normal body weight, preferably from about 10 - 80 mg. In respect of cerivastatin, an entirely synthetic compound, the most appropriate daily dosage is much lower, namely from about 0.1 - 0.8 mg. In the combination therapy of the invention, and afterwards, these dosages may be reduced.

WHAT IS CLAIMED IS:

1. A combination treatment for slowing or arresting the progression and/or effecting the regression of atherosclerotic plaque deposits in a mammalian patient, said combination treatment including the administration to the patient of a cholesterol lowering drug and the administration to the patient of an aliquot of a patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light.

2. The use of an aliquot of a patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light; and a cholesterol-lowering drug, for reducing serum lipid levels and/or combating development of atherosclerosis in a mammalian patient.

3. A process for enhancing the reduction in serum lipid levels in a mammalian patient caused by administration of a cholesterol-lowering drug, which comprises administering to the patient an aliquot of the patient's own blood which has been treated ex vivo with one or more stressors selected from an oxidative environment, thermal stress and UV light and administering to a patient a cholesterol-lowering drug.

4. The invention as claimed in any preceding claim wherein the cholesterol-lowering drug is a statin drug.